phosphotyrosine to EGFR protein for wt and mutant receptors. C) Ratio of phospho-MAPK to total MAPK protein.

[0082] FIGURE 14A-14C. Dose-response of EGFR activation in CR2 mutants. Cells expressing the wt or CR2-mutant receptors were rendered quiescent by growth factor and serum withdrawal, then exposed to control buffer or to increasing concentrations of EGF (0.03 to 100nM). **A**): total cell lysates were analyzed by SDS/PAGE on 4-12% gradient gels, followed by immunoblotting with anti-phosphotyrosine, anti-EGFR or anti-phospho-MAPK antibodies. **B**) and **C**): the films were scanned for densitometric quantitation of the reactive bands and the phospho-Shc and phospho-MAPK data were plotted as % maximal band intensity against EGF concentration. Symbols are: closed circles, wtEGFR; dark triangles, $D^{563}H$-EGFR; light triangles, $V^{583}D$-EGFR; open squares, $E^{578}C$-EGFR.

[0083] FIGURE 15. Mitogenic response to EGF of BaF/3 cells expressing wt or mutant EGFR. [^{3}H]Thymidine incorporation in cells treated with control buffer (open circles) or increasing concentrations of EGF (filled circles) was determined as described in Experimental procedures.

[0084] FIGURE 16. Comparison of mAb528 and mAb806 antibody binding to BaF/3 cells expressing EGFR lacking the CR1-loop. Cells expressing the wt, Δ2-7 or Δ-CR1-loop EGFRs were stained with either mAb528 (dark line) or mAb806 (filled grey) as described in FIGURE2, and analysed on a FACScan. The median fluorescence channel for each peak was determined using the statistical analysis software in CellQuest and used to calculate the ratios between the two antibodies. Control fluorescence of an irrelevant, class- matched antibody is presented as a dotted line overlay.

11/16/05

[0085] FIGURE 17A-17E. EGFR conformations and activation. The EGFR undergoes a major conformational change during the transition from the low affinity to the high affinity state. The low affinity conformation (**A**) is tethered by intra-molecular interactions between the two cysteines-rich domains CR1 and CR2. The tethered monomer (**A**) is in equilibrium with either the tethered dimer (**B**) or a high affinity untethered monomer (**F**). It appears that transmembrane (TM) and/or kinase domains drive the formation of both the tethered dimmer